FACTORS CONTROLLING APTITUDE AND PHAGE DEVELOPMENT IN A LYSOGENIC *ESCHERICHIA COLI* K-12*

by

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The discovery by LWOFF, SIMINOVITCH AND KJELDGAARD[1] of the induction of phage formation in lysogenic organisms by ultraviolet irradiation offers an unusual opportunity for the study of the factors which control the development of a virus. For, unlike in studies of extrinsic phage-host relationships, interpretations of experiments with lysogenic organisms need not be qualified by considerations of phage adsorption and penetration. The shock of ultraviolet irradiation precipitates an intracellular chain of events which culminates in the liberation of phage particles from the bursting cells.

However, it must be borne in mind that any nutritional or metabolic stress can affect a lysogenic organism at two different stages in the process of phage reproduction. Both the "aptitude" of an organism—*i.e.* the ability to respond to the ultraviolet irradiation by phage formation—and the development of prophage into bacteriophage after the inducing shock, may be independently affected.

The effect of nutritional factors on aptitude in *Bacillus megatherium* has been previously demonstrated[1,2]. It was found that the dose of ultraviolet irradiation necessary to induce bacteriophage development in 100% of the bacteria was considerably higher if the bacteria had been grown in a synthetic medium than if grown in the presence of yeast extract. Moreover, in the absence of a carbon source the aptitude decreased.

The effect of glucose starvation on the aptitude of another lysogenic organism, *Pseudomonas pyocyanea*, has been studied by JACOB[3] who has found that such starvation prior to irradiation decreased the aptitude of this organism as well[3]. The aptitude was restored if, after a glucose starvation lasting for as long as 12 hours, glucose was provided to the organism ½ hour prior to irradiation.

The effect of glucose starvation after irradiation is different in these two lysogenic organisms. In *B. megatherium* such starvation suppresses phage development. However *P. pyocyanea* can be starved of glucose for as long as twelve hours after irradiation and still, if growth is restored by the addition of glucose, bacteriophage are produced.

These findings have demonstrated the importance of the nutritional condition of the organism to aptitude and phage development.

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It seemed of interest to investigate the relation between some aspects of nitrogen metabolism on aptitude on one hand, and on phage development on the other. Lysogenic organisms which have synthesizing deficiencies appeared to be promising objects of such studies. WEIGLE AND DELBRÜCK[4] have shown that ultraviolet irradiation produces lysis and phage production in *E. coli K-12*, an organism which had been found to be lysogenic by LEDERBERG. Two deficient mutants of K-12, isolated by LEDERBERG, were available to us. Mutant W33 (T.L.B_1-) is deficient in its ability to synthesize threonine, leucine and thiamin, while mutant Y87 (M.B.-) is deficient in the synthesis of methionine and biotin.

The effect of specific amino acid starvation on aptitude and on bacteriophage development and the effect of nitrogen starvation on aptitude is presented here.

EXPERIMENTAL

The synthetic culture medium for the wild type strain K-12 was as follows: salt solution "56"[5] 25 ml, solution of "micro" elements[6] 4 ml, 30% glucose solution 1 ml, water to 100 ml.

For mutant Y87 (M.B.-) the above medium was supplemented with 1 μg of biotin and 1500 μg of DL-methionine per 100 ml of solution. For mutant W33 (T.L.B_1-) the stock solution was supplemented with 100 μg of thiamin, 10 mg of L-leucine and 100 mg of DL-threonine per 100 ml of solution.

Two methods are available for the starvation of microorganisms of specific nutrients. Growth on limiting amounts of nutrient, or centrifugation in the exponential growth phase, followed by washing and resuspension in a deficient medium. To achieve nitrogen starvation in the wild type strain both methods were used. In the case of the mutant strains, where there is a possibility of selection, or, of back-mutation to non-exacting strains, short periods—three hours—of suspension in deficient media were employed. That the accumulation of non-exacting organisms during such periods is negligible is apparent from the lack of measurable growth without the specific nutrients.

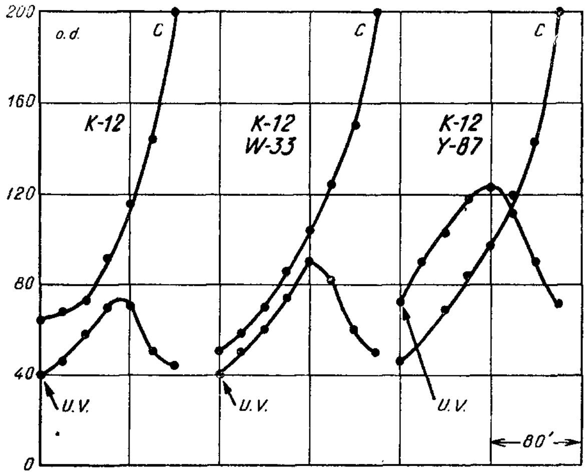

Fig. 1. Growth curves of normal and irradiated organisms. The irradiation was 500 ergs/mm² in each case

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Growth and lysis were assayed by the determination of optical density. That the release of phage particles accompanies lysis was determined in some experiments by plating the lysates with a sensitive organism, *E. coli K-12 S.* (We are indebted to Dr J. WEIGLE of the California Institute of Technology for this strain which had been isolated by J. LEDERBERG).

The ultraviolet irradiations were carried out with a high pressure low voltage quartz lamp using the techniques previously described[1]. The energy to which the organisms had been exposed varied from 333 to 1000 ergs per mm^2.

RESULTS AND DISCUSSION

In Fig. 1 the normal growth curves of K-12, K-12 Y87 and K-12 W33 and those obtained after ultraviolet irradiation are presented. It is apparent that the wild type strain as well as the mutants undergo lysis as the result of ultraviolet irradiation.

1. *The effect of nitrogen and amino acid starvation on aptitude*

In Fig. 2 growth curves of the mutant strains which have been deprived of the amino acids essential to them for three hours prior to the ultraviolet irradiation, and the growth curve of the wild type organism which has been deprived of nitrogen for three hours prior to the irradiation are presented. In each case it was found that such starvation produced a decrease in aptitude: irradiations which normally induce lysis and phage formation were without effect on the growth curves.

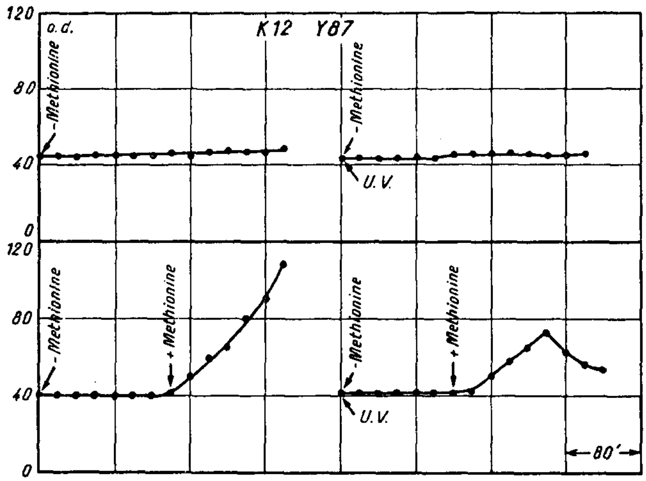

Fig. 2. The effect of starvation prior to irradiation on aptitude. The irradiation was 660 ergs/mm^2 in each case. The lacking nutrient was added immediately after the irradiation

The irradiation could be increased on these starved organisms by 30%, without obtaining any change in the growth curves. When the irradiation was increased a 100% some plateaus in the growth curves were observed. However, high dosages of irradiation inhibited growth, as well, and therefore such experiments are difficult to assess. To rule out the possibility that the lack of lysis in organisms starved prior to the irradiation is due merely to a diminished metabolism, the following experiment was performed.

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Normal organisms were kept at 3° for as long as 20 hours and were then irradiated at 3°. These organisms, when permitted to resume growth at 37°, lysed in the same manner as those which were in exponential growth at the time of irradiation.

It was also found that glucose starvation prior to irradiation affects the aptitude of K-12 in the same manner as that of *B. megatherium* and *P. pyocyanea*. After 3 hours of glucose starvation of K-12 an irradiation which would have induced phage formation in normal bacteria had no effect on the growth curve: there was no measurable lysis. It is apparent that aptitude is sensitive to a variety of different starvations.

2. The effect of amino acid starvation on bacteriophage development

In Fig. 3 growth curves of Y87 (M.B.–) which had been deprived of methionine after the ultraviolet irradiation are presented. Upon the addition of methionine, 120 minutes after the irradiation, growth was resumed and, 100 minutes later, lysis took place. Thus the timing of the lysis was determined by the addition of the methionine,

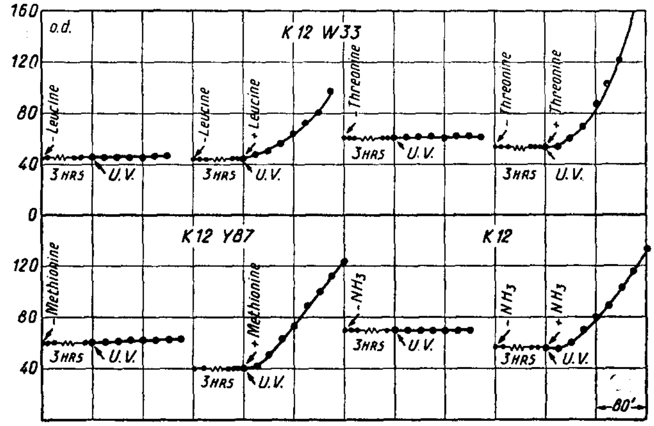

Fig. 3. The effect of starvation after irradiation on phage development. After irradiation the organisms were placed in a medium devoid of methionine. The iradiation was 500 ergs/mm² in each caes

indicating that apparently no development of phage took place during the interval of methionine starvation after the irradiation. It was also found that glucose starvation after irradiation had the same effect on *E. coli* K-12 as on *P. pyocyanae*. The organisms were placed in a glucose free medium for three hours after the u.v. shock. Upon the addition of glucose, growth was resumed and, 80 minutes later, lysis ensued. While starvation of the organism prior to irradiation decreased the aptitude, once the organism was subjected to the irradiation, subsequent starvation of methionine or of glucose did not suppress the effect of the inducing shock.

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SUMMARY

1. The effect of starvation on the aptitude and phage development in *E. coli* K-12 has been studied.

2. Starvation of glucose, of specific amino acids, or of nitrogen before irradiation produces a diminution of aptitude.

3. Starvation of the organism of glucose or of an essential amino acid—methionine—after irradiation does not suppress the effect of induction: Bacterial growth and lysis are resumed after the addition of the lacking metabolite.

RÉSUMÉ

1. Nous avons étudié l'effet d'un régime incomplet sur l'"aptitude" et le développement des phages chez *E. coli* K-12.

2. Une carence en glucose, en acides aminés spécifiques ou en azote avant l'irradiation produit une diminution de l'aptitude.

3. Si l'organisme est privé de glucose ou d'un acide aminé essentiel — méthionine — après l'irradiation, l'effet de l'induction n'est pas supprimé: La croissance bactérienne et la lyse reprennent après adjonction des métabolites qui avaient fait défaut.

ZUSAMMENFASSUNG

1. Der Einfluss einer mangelhaften Ernährung auf die "Aptitude" und die Bakteriophagen-Entwicklung bei *E. coli* K-12 wurde untersucht.

2. Mangel an Glucose, an spezifischen Aminosäuren oder an Stickstoff vor der Bestrahlung setzt die "Aptitude" herab.

3. Mangel an Glucose oder an notwendigen Aminosäuren — Methionin — nach Bestrahlung unterdrückt den Induktionseffekt nicht: Bakterienwachstum und Lyse beginnen von Neuem nach Zugabe der fehlenden Metabolite.

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